UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT 30 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Terbutryn - Review Mutagenicity Studies Submitted under EPA Accession No. 402814-01, -02, and -03 in Response to the Registration Standard
EPA Registration No. 100-540

TB Project No.: 7-0959
Caswell No.: 125D

FROM: Irving Mauer, Ph.D. 10/29/87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor/James R. Yowell, PM Team 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Head 10/30/87
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C) 10/30/87

Registrant: Ciba-Geigy Corporation, Greensboro, NC

Request

Under a cover letter dated July 30, 1987, the registrant (Ciba-Geigy Corporation) submitted three mutagenicity studies in order to comply with the Registration Standard requirement for mutagenicity and as a result of discussions at a meeting held July 23, 1987 with EPA/TB. Further, under separate cover, Ciga-Geigy plans to request a waiver of the requirement for a sister-chromatid exchange study.

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TB Conclusions/Recommendations

The three studies submitted and the Toxicology Branch (TB) assessments are as follows:

1. Terbutryn Technical Salmonella/Mammalian Microsome Mutagenicity Assay (Ames Assay), Report #87024 (Min #872186), June 18, 1987, performed at the Pharmaceuticals Division, Ciba-Geigy, Summit, New Jersey.

   TB Evaluation: ACCEPTABLE, and negative (nonmutagenic)

2. Salmonella/Mammalian - Microsome Mutagenicity Test (OECD-Conform), Report #860706, March 1987, performed at Ciba-Geigy, Ltd., Basle, Switzerland.

   TB Evaluation: ACCEPTABLE, and negative (nonmutagenic)

3. Autoradiographic DNA Repair Test on Rat Hepatocytes, Report #850909, July 24, 1986, performed at Ciba-Geigy, Ltd., Basle, Switzerland.

   TB Evaluation: ACCEPTABLE, and negative for DNA repair (UDS)

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TOXICOLOGY BRANCH: DATA REVIEW

Reviewed by: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

TB Project: 7-0959

Date: 10/29/87

Through: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division

10/30/87

Chemical: Terbutryn

Caswell: 125D
EPA Chem: 080813

Study Type: Mutagenicity - Gene Mutation in Bacteria (Salmonella-Ames Test)

Citation: Salmonella/Mammalian - Microsome Mutagenicity Assay (Ames Assay) (Min 872186)

Accession No.: 402814-01

MRID: N/A

Sponsor: Agricultural Division, Ciba-Geigy, Greensboro, NC

Testing Lab.: Ciba-Geigy, Division of Toxicology and Pathology, Summit, NJ

Study No.: 87024 (Min 872186)

Study Date: June 18, 1987

TB Conclusions/Evaluation:

ACCEPTABLE. Nonmutagenic both in the absence and presence of an S9 activation system up to toxic concentrations.

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DETAILED REVIEW

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Test Material:

Terbutryn technical (Batch No. FL870004), purity unstated, supplied as a powder and dispensed neat, dissolved in dimethylsulfoxide (DMSO) for testing.

Procedures:

Cultures of Salmonella strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 were exposed iln triplicate in two separate assays to the test material at concentrations of 0 (DMSO control), 10, 50, 150, 300, and 600 µg/plate, both in the absence and presence of a commercial S9 liver microsomal fraction prepared from Aroclor 1254-induced rats (obtained from Bionetics, Charleston, SC), plus generating NADP co-factors. Confirmed mutagens appropriate to each strain were run concurrently with each test.

Results:

A preliminary cytotoxicity test in TA 100 reported that concentrations of 900 µg/plate were toxic (Report Table 8.1), resulting in 50% reduction in mean revertents and a drastic reduction in mean colony-forming units (CFU/plate) to 4 (vs. 200 for the DMSO vehicle control). An inhibitory effect was also recorded at 600 µg/plate, resulting in a mean CFU/plate of 7 (vs. 200 for the DMSO control).

In both assays, there was no evidence of any increase over DMSO control in the number of revertents in any strain treated with terbutryn with or without activation. Positive controls, sterility checks, strain characterization, etc., employed to assure that the test system was functioning properly, all produced results within expected limits and responses. Mean spontaneous mutation frequencies for all tester strains were within established (referenced) limits.

The authors concluded that, based upon their established criteria, terbutryn technical (FL 870004) was not mutagenic in Ames testing.

TB Evaluation:

ACCEPTABLE, and negative up to toxic concentrations.

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TOXICOLOGY BRANCH: DATA REVIEW

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Toxicology Branch
Hazard Evaluation Division

TB Project: 7-0959

Date: 10/29/87

Through: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division

JWH 10/30/87

Chemical: Terbutryn

Caswell: 125D
EPA Chem: 080813

Study Type: Mutagenicity - Gene Mutation in Bacteria (Salmonella-Ames Test)

Citation: Salmonella/Mammalian - Microsome Mutagenicity Test (OECD-Conform) (Induction of Liver Enzyme Activity with the Test Substance)

Accession No.: 402814-02

MRID: N/A

Sponsor: Agricultural Division, Ciba-Geigy, Greensboro, NC

Testing Lab.: Experimental Pathology Labs, Ciba-Geigy
Basle, Switzerland

Study No.: 860706

Study Date: March 1987

TB Conclusions/Evaluation:

ACCEPTABLE. Nonmutagenic in terbutryn-activated cultures.

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DETAILED REVIEW

Test Material:

Terbutryn technical (aka GS 14260 tech), Batch No. P506001, 97.4% ai, dissolved in dimethylsulfoxide (DMSO) for testing.

Procedures:

Cultures of Salmonella strains TA 98, TA 100, TA 1535, and TA 1537 were exposed to a special microsomal (S9) activation system (only), and the test substance at concentrations of 0 (DMSO control), 20, 78, 313, 1250, and 5000 µg/plate in both standard plate as well as preincubation assays. The liver microsome activation system was prepared from livers of rats pretreated with terbutryn technical administered orally at daily doses of 150 mg/kg for 14 consecutive days. Standard mutagens appropriate to the activated bacterial strains served as positive controls: 250 µg cyclophosphamide (TA 1535) and 5 µg 2-aminoanthracene (TA 98, TA 100, TA 1537). In toxicity testing with unactivated TA 100, 4-nitroquinoline-N-oxide was the positive control.

Results:

In toxicity testing performed with nonactivated TA 100, no growth inhibition was found at the highest concentration, 5000 µg/plate, consequently this concentration was selected as the top dose in the main mutagenicity assays.

Neither plate tests not preincubation tests gave any indication of any increased incidence of revertents to histidine-prototrophy, compared to solvent control. Inhibitory effects were evident at the two highest doses, 1250 and 5000 µg/plate, especially in preincubation tests, and the test substances precipitated in soft agar.

Concurrent control incidences for all strains were consistent with a range of spontaneous revertents collected from 81 previous experiments performed during a 12-month period in 1985.

The authors concluded that terbutryn-activated cultures of standard Ames strains were negative for mutagenicity.

TB Evaluation:

ACCEPTABLE. This novel approach of employing the test compound to test for mutagenic metabolites gave negative results, i.e., terbutryn was nonmutagenic in both standard plate-incorporation as well as preincubation tests.

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Hazard Evaluation Division

JWH 10/30/87

Chemical: Terbutryn

Caswell: 125D
EPA Chem: 080813

Study Type: Mutagenicity - DNA Damage/Repair (UDS/Rat HPC)

Citation: Autoradiographic DNA Repair on Rat Hepatocytes

Accession No.: 402814-03

MRID: N/A

Sponsor: Agricultural Division, Ciba-Geigy, Greensboro, NC

Testing Lab.: Experimental Pathology Labs
Ciby-Geigy, Basle, Switzerland

Study No.: 850909

Study Date: July 1986

TB Conclusions/Evaluation:

ACCEPTABLE. Nongenotoxic for UDS (DNA repair) up to cytotoxic concentrations.

DETAILED REVIEW

Test Material:

Terbutryn technical (aka GS 14260 tech), Batch No. P506001, 97.4% ai, dissolved in dimethylsulfoxide (DMSO) for testing.

Procedures:

Cultures of fresh hepatocytes isolated from an adult male rat induced with Aroclor 1254 (viability > 90%) were allowed to attach to coverslips over a 2-hour period, cultivated overnight (to permit adhesion), and then exposed to 0.25, 1.25, 6.26 and 31.3 µg/ml test material (based upon a preliminary toxicity test). Parallel cultures were exposed to the mutagen 4-aminobiphenyl (50 µm ABP), as well as to the vehicle (DMSO), while another was left untreated.

Immediately after addition of test material (or control substances), tritiated thymidine was added, and cultures incubated 5 hours, following which cultures were fixed and prepared for autoradiography employing conventional methods. After 6 days' exposure to the photographic emulsion, slides were stained with hemotoxylin-eosin and silver grains counted over both background cytoplasmic areas and nuclei, and data expressed as mean net grains from a total of 150 cells per experimental point (50 cells per slide). [Mean net nuclear grain count = total number grains over nuclei less average grain count over three nuclear-size cytoplasmic areas.]

Results:

From the seven concentrations tested in the preliminary toxicity assay (15.6 to 1000 µg/mL), concentrations of 62.5 µg/mL and above were toxic (< 1% viable cells); 31.3 µg/mL, therefore, was the highest applicable concentration in the main assay.

At all concentrations in the main repair assay, the mean net nuclear grain count in test cultures did not differ from the vehicle control (0.08 to 0.30 in test cultures vs. 0.23 in DMSO cultures, and 0.16 in untreated cultures). By contrast, 4-ABP cultures manifested 9.10 net grains per nucleus.

The report contains historical control values from studies done within a recent 12-month period (as Report Table 6), as well as a complete set of raw data.

The authors concluded there were no marked deviations between any treatment group and vehicle control, i.e., the test material was not genotoxic under the conditions of this repair assay.

TB Evaluation:

ACCEPTABLE, and negative for UDS repair up to cytotoxic concentrations.

END